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Determination of Fenvalerate in Flowing-Seawater Exposure Studies

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16. ABSTRACT

An analytical method is reported for the quantitiative determination of pydrin (fenvalerate) suitable for the detection of small amounts of this compound in flowing-water bioassays. The method utilizes the retention of pydrin on Sep-Pak C_{18} cartridges, subsequent elution and re-extraction, clean-up, and gas chromatographic analysis using capillary columns. By considering factors such as, sample size and instrument sensitivity, the limit of detection approached 0.02 ng/l of pydrin in seawater.

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Determination of Fenvalerate in Flowing-Seawater Exposure Studies

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Fenvalerate (Pydrin)[2] is a pyrethroid which when present in the water at low concentrations appears to be highly toxic to crustaceans. Reported 96-h LC-50 values are 0.14 µg/L for lobster (Homarus americanus) and 0.04 µg/L for shrimp (Callinectes septemspinosa) in static tests (MCLEESE et al. 1980) and 0.002 µg/L for grass shrimp larvae (Palaemonetes pugio) in flow-through tests (TYLER MCKENNEY, pers. comm.). Since the 0.002 µg/L value was based on nominal rather than measured concentrations, and established methods were not sensitive enough, (BUCK et al. 1980; CHAPMANN HARRIS 1978; HILL 1981; LEE et al. 1978; PAPADOPOULOU-MOURKIDOU et al. 1980; WSZOLEK et al. 1980; HORIBA et al. 1980a,b; HORIBA et al. 1980), a method for the analysis of fenvalerate at low concentrations in seawater was developed.

Fenvalerate has two centers of chirality and therefore exists as a mixture of four stereoisomers, two of which are diastereoisomers, the other two are the corresponding enantiomers.

METHODS

Two different dosing systems were used to introduce fenvalerate into seawater. One system used direct infusion of the various fenvalerate stock solutions via a syringe pump (fig. 1), and the other system produced the desired concentrations via a serial diluter (SCHIMMEL et al. 1975) (fig. 2). Both systems were all glass, designed to deliver seawater containing identical amounts of the carrier triethylene glycol. The diluter provided 1 L of each final concentration of fenvalerate in seawater every 3 min, while the syringe pumps continuously discharged into the incoming seawater syphon which was adjusted to a flow rate of 25 1/h of seawater.

A Gas chromatograph (Hewlett-Packard 5880A) equiped with ^{63}Ni electron-capture (EC) and nitrogen/phosphorus thermionic (NP) detectors was used for the detection and quantitation of both the diastereoisomers of fenvalerate (HORIBA et al. 1980a,b; HORIBA et al. 1980). A glass column (4mm X 50 cm, 3% OV-101) was used for

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[2]Mention of trade names and commercial products does not constitute endorsement by the Environmental Protection Agency.

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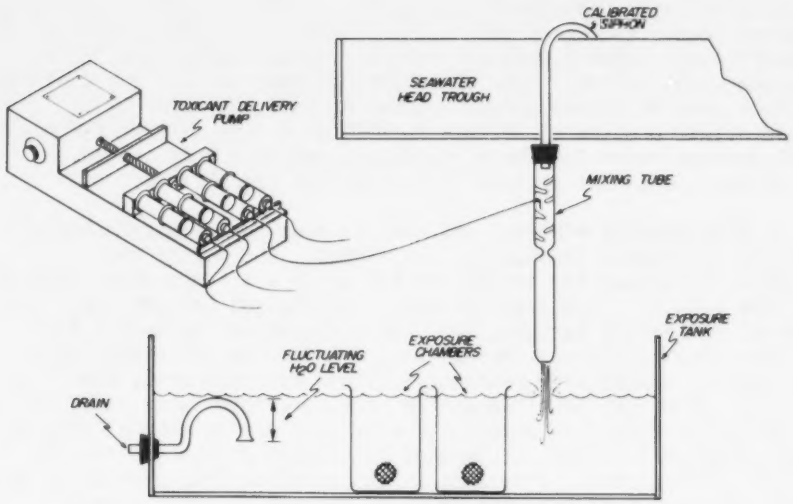

Fig. 1 Syringe pump system.

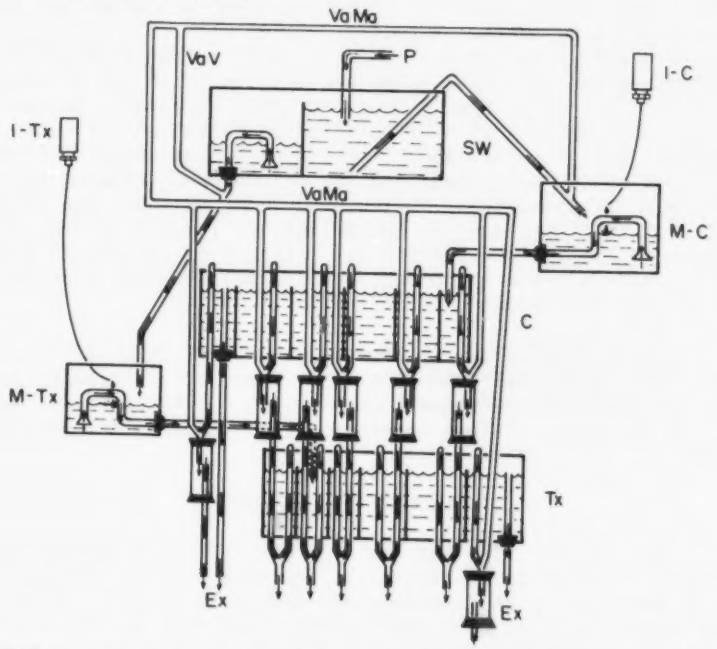

Fig 2 Diluter system. C-seawater with carrier; Ex-excess water; I-C-carrier injector; I-Tx-toxicant and carrier injector; M-C-carrier mixing box; M-Tx-toxicant and carrier mixing box; P-seawater pump; SW-filtered seawater cells; Tx-Toxicant, carrier, and seawater cells; VaMa-vacuum manifolds; VaV-vacuum venturi.

sample analysis from the system using a diluter, while a capillary column (Hewlett-Packard WCOT methyl silicon, Carbowax 20M deactivated; 0.2mm X 25m) was used for sample analysis from the syringe pump system. Special conditions observed for the capillary column were 30 second column loading at 150C, followed by a temperature program of 30C/min to 250C at a flow rate of 1-2 mL/min 10% methane/argon for the EC detector, and helium, nitrogen, hydrogen and air at optimal flow rates for the NP detector.

A Milton-Roy miniPump was used to pump the seawater through Waters Associates Sep Pak C_{18} cartridges. The intake was Teflon tubing and the outlet was 0.5 µm ID stainless steel tubing. A flow rate of 5.4 mL/min was easily maintained through the cartridge without backpressure; the effluent was collected for volume determinations. Before use, the Sep Pak cartridges were thoroughly washed with methanol followed by high-purity distilled water. After the sampling period, the cartridges were rinsed with high purity distilled water; the flow used during sample collection was reversed in order to dislodge and remove microorganisms and other particulates. Returning to the original flow direction, the fenvalerate was eluted with 10 mL of methanol. After solvent evaporation to 1 mL under nitrogen at 30C in 15-mL conical centrifuge tubes, 1 mL of high-purity water was added and the resulting solution was extracted 3 times with 3-mL portions of hexane; centrifugation was used to break any emulsions that formed. The hexane was then evaporated under nitrogen at 30C to about 0.5 mL. Florisil columns were prepared using disposable Pasteur pipets with glass wool plugs, filled to height of 5 cm with Florisil activated at 145C. A layer of 0.5 cm sodium sulfate also stored at 145°C was added, and the columns were cleaned by rinsing with 5 mL ethyl acetate, followed by 10 mL hexane. The 0.5-mL extract was then placed on the column, followed by a 5 mL of hexane wash. The fenvalerate was eluted from the column with 5 mL of 3% ethyl acetate in hexane, and the solution was concentrated to an appropriate volume under nitrogen at 30C.

Standard solutions of fenvalerate (Shell Chemical Co., SD437754, commercial grade) in iso-octane (2,2,4-trimethylpentane) were prepared at concentrations of 1.12, 0.112, and 0.011 µg/µL for GC analysis. Injection volumes for standards and samples were 3 µL.

RESULTS AND DISCUSSIONS

The capacity of a Sep-Pak C_{18} cartridge to quantitatively contain fenvalerate was tested by applying 5 µg of fenvalerate in 50 µL methanol in one instance and 500 pg in 50 µL of methanol in the other, followed by a rinse with 2 L of high purity water. Using the analytical procedure outlined, the recovery was >95% in both cases. Since these amounts adequately bracket the experimental values, methanol elution of fenvalerate from Sep-Pak C_{18} was deemed a proper technique by which large volumes of water can be extracted.

Analysis of fenvalerate after the hexane extract from water/methanol was difficult at the lower concentrations because of large amounts of interfering materials. Late-eluting peaks which orginated from the Sep-Pak cartridges were largely removed by the methanol wash. The most seriously interfering peaks came from the inadvertent extraction of algae caught in the C_{18} matrix, since they could not be removed because of pydrin adsorption to any filter used during such a process. The methanol used to extract the fenvalerate from the C_{18} packing lysed the algae cells and solubilized most of the pigments. A Florisil cleanup step was thus added to remove all pigmented materials and much of the remaining late-eluting materials. Fig. 3 shows the separation obtained when using a regular GC column, 4 mm ID glass packed with 3% OV-101. While this method gives reasonable results at higher concentrations, contaminant interference at <0.5 ng/L of fenvalerate was much too high as seen by the standard deviations in table 1. In order to improve the minimum detectability, a capillary column system was used. To optimize conditions, the splitless mode of injection was chosen using a WCOT methyl silicon, carbowax 20M™ deactivated (25m, 0.2 mm ID) column. Under the conditions described earlier (see Methods), the H-P 5880A column inlet configuration in the splitless mode allows the sample to vaporize at 280C (inlet temp.), and, while sample sweeps into the column for 30 sec, the fenvalerate condenses at the head of the column held at 150C. A temperature increase then sweeps the compound through the column effecting the separation shown in fig. 4.

It is apparent from tables 1 and 2 that the concentrations of fenvalerate in the water are much less than their nominal concentrations, ranging from as high as 40% at the inlet and as low as 10% at the outlet in the case of "3.2 ng/L", to as low as 10% at inlet and outlet for "0.8 ng/L". While this does not constitute a trend, it attests most probably to the strongly adsorptive nature of fenvalerate. Attempts were made in all cases to confirm the presence of fenvalerate with a nitrogen/phosphorus thermionic detector. However, the sensitivity of this detector was useful only for extracts prepared from water with concentrations of >0.3 ng/L with regular columns and >0.6 ng/L with capillary columns. The standard deviations listed in tables 1 and 2 reflect sample extraction recovery (>95%), cleanup (90%), evaporative loss (5%), measurement of final volume (up to 20%) and detector noise and peak interference (up to 50%). The last two were the major contributors to errors (the others being relatively small and constant) and depended much on the size of the final volume containing the fenvalerate versus detector sensitivity. The use of capillary columns reduced the error contribution due to detector noise and peak interference (up to 20%). However, the necessity of having higher final concentration and, hence, lower volumes, raised the error contribution due to volume measurement. The lower sensitivity of the N/P detector with capillary columns is, of

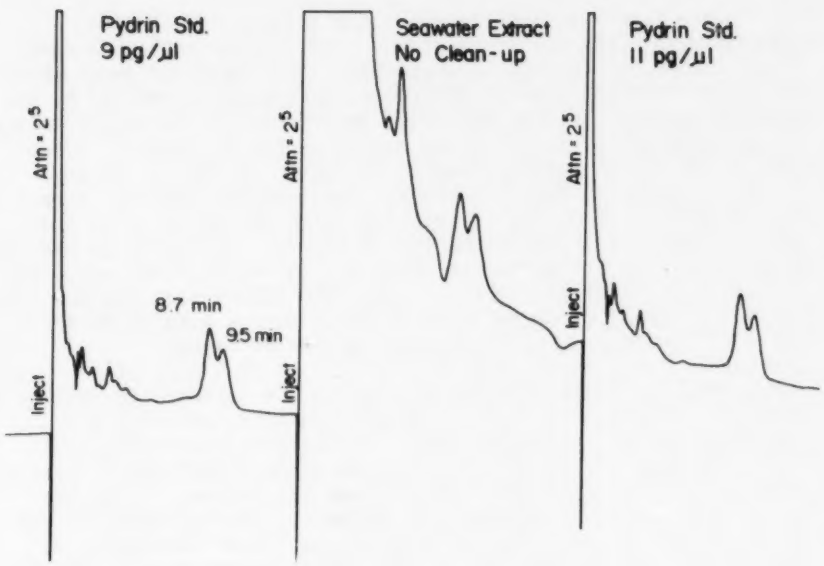

Fig. 3 GC trace of fenvalerate using OV-101 glass column. See Methods for details.

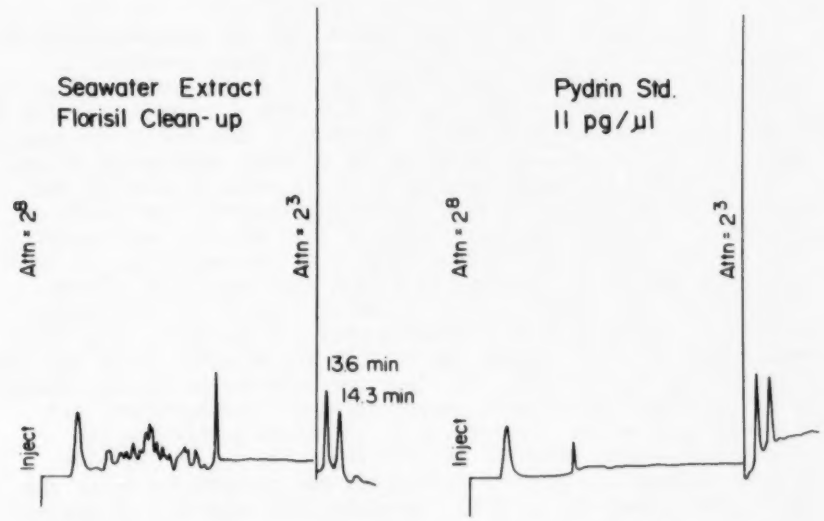

Fig. 4 GC trace of fenvalerate using a capillary column. See Methods for details.

Table 1. Residues of pydrin in seawater as determined by packed-column gas chromatography.

System Delivery (days)	Nominal Conc. (ng/L)	Volume Collected (L)	Sampling Location	Measured Conc. (ng/L)±SD	Method of Analysis	
					calculation/confirmation	
16	3.2	5.2	outfall	0.36 ± 0.10	NP	EC
23	3.2	2.3	outfall	0.69 ± 0.20	NP	EC
29	3.2	5.2	outfall	0.64 ± 0.19	NP	EC
36	3.2	5.4	outfall	0.52 ± 0.22 0.32 ± 0.16	EC NP	
42	0.8	6.4	outfall	0.15 ± 0.12	EC	no
44	0.8	5.8	outfall	0.07 ± 0.05	EC	no
	seawater control	4.9		N.D.	EC	
	Solvent control			N.D.	EC	

[1] Below detection level for NP

NP - Nitrogen/Phosporus detector

EC - Electron-capture detector

Table 2. Residues of pydrin in seawater as determined by capillary column gas chromatography.

System Delivery (days)	Nominal Conc. (ng/L)	Volume Collected (L)	Sampling Location	Measured Conc. (ng/L) ± SD	Method of Analysis	
					calculation/confirmation	
12	3.2	1.9	inlet	1.36 ± 0.15	EC/C	NP/C
15	3.2	2.4	inlet	1.20 ± 0.13	EC/C	NP/C
16	3.2	6.4	outlet	0.36 ± 0.09	EC/C	no[1]
21	3.2	6.6	outlet	0.28 ± 0.08	EC/C	no
23	3.2	2.5	outlet	0.26 ± 0.08 0.19 ± 0.15	EC/C EC	no no
24	3.2	5.4	outlet	0.28 ± 0.09 0.58 ± 0.21	EC/C EC	no no
34	3.2	7.0	outlet	0.51 ± 0.15	EC/C	no
35	0.8	8.9	inlet	0.12 ± 0.06	EC/C	no
36	0.8	10.2	inlet	0.04 ± 0.02	EC/C	no
37	0.8	7.7	inlet	0.02 ± 0.01	EC/C	no
	seawater control	6.2		N.D.	EC/C	no
	Solvent control			N.D.	EC/C	no

[1] Below detection level for NP/C

EC/C Electron-capture/capillary (EC/C)
NP/C Nitrogen-phosphorus/capillary

course, due to the fact that smaller amounts of fenvalerate are seen because of the fixed injection volume (see Methods).

The pydrin concentrations shown in table 1 were found in a seawater exposure system in which the toxicant was introduced by a diluter (Fig. 2) in order to comply with standard procedure. The concentrations shown in table 2 were obtained in a system using syringe pumps (Fig. 1).

No clear trend emerges from tables 1 and 2 with regard to differential amounts of fenvalerate delivered to the test chambers by the two systems, which can, however, be misleading since the concentrations reveal nothing about the availability of the toxicant to the animals (SCHOOR 1975). In the long pathway of the diluter, microorganisms and other particulate matter may adsorb the fenvalerate, and, while it can be desorbed during the analytical procedure, it may not be available to the test animals unless ingested during feeding. Since adsorption is a time dependent phenomenon, it may not occur to as large a degree when the toxicant is injected directly into the test chambers where it has a comparatively short residence time, and a certain but larger amount could be "bio-available" to the organisms tested in this system. Differences between the two systems can currently be demonstrated only by toxic effects since the chemical analysis cannot differentiate between fenvalerate in true solution and fenvalerate adsorbed to particulate matter.

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